Recent Advances in Pharmaceutical Science Research
www.rapsr.com
Vol-1 | No-1 | 2012 | 60-70

Original Research Article

Kinetic spectrophotometric determination of fluoroquinolines in pharmaceutical formulations

Virupaxappa BS*[1], Raviraj M. Kulkarni[2], Girish Bolakatti[3], Shivaprasad KH[1] and Latha M.S.[4]

[1] Department of Chemistry, VSK University,Bellary, Karnataka, (INDIA)
[2]Department of Chemistry, Gogte Institute of Technology, Udyambag, Belgaum, Karnataka, (INDIA)
[3]Department of Pharmaceutical Chemistry, Bapuji Pharmacy College, Davangere, Karnataka (INDIA)
[4] Department of Chemistry, GM Institute of Technology, Davangere, Karnataka, (INDIA)

Abstract

Kinetic spectrophotometric methods were developed for determination of Fluoroquinolones namely ciprofloxacin (CIP), Norfloxacin (NOR) and Pefloxacin (PF) in a pharmaceutical preparations. The methods are based on oxidation of Floroquinolone drugs with potassium permanganate in acidic media and measurement of the enhancement in the absorbance of manganate ion at 526 nm by spectrophotometry. The calibration graphs were constructed using the rate constant and fixed time methods. The linearity ranges for CIP, NOR and PF were found to be 33.1-331 µg/ml, 31.9-319µg/ml and 33.5-335µg/ml respectively. The procedures were applied successfully for determination of Fluoroquinolone drugs in commercial tablets. The proposed methods can be recommended for routine analysis of Fluoroquinolones in QC laboratories.

Keywords: Kinetic spectrophotometry, Fluoroquinolones, Ciprofloxacin, Norfloxacin, Pefloxacin and $KMnO_4$.

***virupaxb@gmail.com, ravirajmk@git.edu**

1.0 Introduction

Fluoroquinolones are the class of broad-spectrum antibiotics, which are active against both gram-positive and gram-negative bacteria. The parent compound of all Fluoroquinolones is nalidixic acid. Fluoroquinolones are widely used to treat human and veterinary diseases [1,2]. Fluoroquinolones can enter cells easily and therefore are often used to treat intracellular infections. They are extremely useful for the treatment of a variety of infections, including urinary tract infections, soft tissue infections, respiratory infections, bone-joint infections, typhoid fever, sexually transmitted diseases, acute bronchitis and sinusitis.

Ciprofloxacin (CIP- 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-piperzine-1-yl-quinoline-3-carboxylic) is a broad spectrum antibiotic. Several spectrophotometric methods were developed for the determination of Ciprofloxacin [3-8], ciprofloxacin is a second generation antibiotics with an expanded spectrum of activity against and gram positive and gram negative bacteria [9].

Norfloxacin, 1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid is a synthetic broad spectrum antibacterial drug. The drug and its formulations are listed in the United States pharmacopeia [10] and European pharmacopoeia [11]. Other techniques used for its quantification include HPLC [12]. This is also determining by charge transfer reactions [13-14]. The quantification of Norfloxacin [15] was done using Fe (III) in sulphuric acid medium. Two extractive spectrophotometric methods [16] have been reported based on the formation of chloroform extractable ion association complex.

Pefloxacin,[1-ethyl-6-fluoro-7-(4-methyl-1-pipezinyl)-4-oxo-1,4-dihydro-3-quinoline carboxylic acid] is a new synthetic antibiotic from the group of 4-quinolones, which makes possible the successful treatment of serious infections caused by group of bacteria. The drug has a wide antimicrobial spectrum and its oral use as simplified the treatment of infection which had been previously dealt with parenterally [17-20]. The chemical structures of Fluoroquinolones are shown in Figure 1.

2.0 Materials and methods

Apparatus

A Peltier Accessory (Temperature controlled) Varian Cary 50 model UV-Vis spectrophotometer equipped with 1 cm quartz cell was used for all spectral measurements. Systronics pH meter were used for the accurate pH determinations.

Materials and reagents

All the materials were of analytical reagent grade and the solutions were prepared with double distilled water, samples of Ciprofloxacin, Norfloxacin and Pefloxacin were generously supplied by Cipla Pharmaceuticals pvt. Ltd, Goa. Potassium permanganate (Merck, Germany) 0.001 M solution was prepared by dissolving 0.0158 g $KMnO_4$ in 100 ml of double distilled water, followed by boiling and filtration through sintered glass. Potassium permanganate solution should be freshly prepared and its molarity was checked titrimetrically. Sodium hydroxide (Merck, Germany), 2M NaOH was prepared by dissolving 8g of NaOH in 100ml of double distilled water. 2 M perchloric acid was prepared by dissolving 17.5 ml of $HClO_4$ in 100 ml of double distilled water. 2M $NaClO_4$: was prepared by dissolving equal

proportions of 2M NaOH and 2M $HClO_4$. 10 % Acetic acid is prepared by dissolving 10 ml Acetic acid in 100 ml double distilled water.

Preparations of standard solution

A Working standard solution of 0.01 M Ciprofloxacin, Norfloxacin and Pefloxacin were prepared by dissolving 0.331 g, 0.319g, and 0.335g in 100 ml of 10% Acetic acid.

Kinetic procedure for Fluoroquinolones

All kinetic measurements were performed under pseudo first order conditions where Ciprofloxacin, Norfloxacin and Pefloxacin used were at least 10 fold excess over permanganate at a constant ionic strength of 0.4 mol dm^{-3}.The reaction was initiated by mixing previously thermostatted solutions of $KMnO_4$ and Fluoroquinolones Ciprofloxacin, Norfloxacin and Pefloxacin, which also contained the required quantities of $HClO_4$ and $NaClO_4$ to maintain the required acidity and ionic strength respectively. The temperature maintained at 25 ±0.1 Cº. The course of the reaction was followed by monitoring the decrease in the absorbance of $KMnO_4$ at 526nm for Ciprofloxacin, Norfloxacin and Pefloxacin in acidic medium.

Preparation of dosage forms sample solutions

Twenty tablets were weighed and finely powdered. A quantity of the mixed powder equivalent to 100 mg of CIP, NOR, and PEF were transferred into a 100 ml calibrated flask. Dissolved in about 30 ml of acetone and the mixture was shaken for 5 min. The mixture was filtered using Whatman No. 42 filter paper and the filtrate was evaporated to dryness on a water bath. The residue was washed thoroughly several times with water before dissolving it in 10 % Acetic acid. The solution was then transferred into a 50 ml volumetric flask, made up to the mark with 10 % Acetic acid and suitable aliquot was then subjected to analysis using the procedure described under method 2.5 after diluting to 0.01 M solution.

The cream is dissolved in 10 % Acetic acid and then the procedure was continued as described under tablets after diluting to 0.01 M Solution.

3 .0 Result and discussions

Potassium permanganate as strong oxidizing agent has been used in oxidimetric analytical method for determination of many compounds. During the course of the reaction, the valence of manganese changes. The heptavalent manganese ion changes to the green color (Mn VI), while in neutral and acid medium, the permanganate is reduced to color less (Mn II). The behaviour of permanganate was the basis for its uses in its development of spectrophotometric method. The absorption spectrum of aqueous potassium permanganate solution in acidic medium exhibited an absorption band at 526 nm. The different variables that affect the formation of manganate ion were studied and optimized. Calibration graph of various kinetic procedures are given below (Fig 2).

Kinetic procedure of Fluoroquinolones

The rate constant, Fixed time methods and Fixed concentration method were used for determining Fluoroquinolones namely, Ciprofloxacin, Norfloxacin and Pefloxacin, and the best method was chosen based on applicability, the slope of the calibration graph, the intercept and the Correlation coefficient (R^2).

Fixed time method

A pre-selected time (100secs) was fixed and the absorbance was measured for different concentrations of drugs (Table 1). A plot of the absorbance versus the initial

concentration of Fluoroquinolones was drawn, which was linear and could be used as a calibration graph (Fig 3).

The range of the drug concentrations giving the most acceptable calibration graph with the above was 30.2- 302 µg/ml).

Rate constant method

Pseudo-first order rate constants were calculated for Ciprofloxacin, Norfloxacin and Pefloxacin Concentrations in the range from 33.1-331 µg/ml, 31.9-319µg/ml and 33.5-335µg/ml and are presented in (Table 2). A plot of K_{obs} versus [Ciprofloxacin, Norfloxacin and Pefloxacin] is drawn, which was used as a calibration graph.

Fixed concentration method

A preselected value of the absorbance was fixed, and the time was measured for different Drugs concentrations. The range of the drug concentrations giving the most acceptable calibration graph with the above was very limited which could be a disadvantage.

Initial rate method

In this method, graphs of the rate (at the beginning of the reaction) versus the drug concentration were not easy to obtain because the reaction was fast. Thus, the tangents to the curves at zero time were not easy to draw. This method was therefore abandoned.

The best correlation coefficient was obtained for the fixed time method, and the value of the slope was also high. Even though the range was limited compared to the rate-constant method, the Fixed-time method was found to be more applicable.

Statistical analysis of the results in comparison with the official method

The performance of the proposed method was judged by calculating the student t-test and variance ratio F-test. At the 95% confidence level, the calculated t- test and F-values do not exceed the theoretical values, indicating that there is no significant difference between the proposed method and the official method. From an analytical point of view, it is concluded that the described procedure allows for the determination of Fluoroquinolones in pure and pharmaceutical dosage forms. Unlike the spectrofluorometer, as well as gas chromatographic and HPLC procedures, the instrument is simple and inexpensive. Its importance lies in the chemical reaction upon which the procedure is based, rather than sophistication of the instrument. This aspect of the kinetic method of determination is of major interest in analytical pharmacy, since it offers a distinct possibility for the assay of a particular component in complex dosage formulations.

Validation of the proposed method

Concentration range is established by confirming that the analytical kinetic procedures provides a suitable degree of precision, accuracy and linearity when applied to the sample containing the amount of analyte within or at the extreme of the specified of the range of the analytical procedure. In this work, concentrations ranging from 33.1-331 µg/ml, 31.9-223.3µg/ml and 33.5-335µg/ml were studied for the investigated drugs in the Rate constant method and concentration ranging from 33.1-331 µg/ml, 31.9-319µg/ml and 33.5-335µg/ml were studied for the investigated drugs in the constant time method (at preselected fixed time for 100 secs).The whole sets of experiments were carried out through this range to ensure the validation of the proposed procedure. Linear calibration graphs were obtained for all the studied drugs by plotting the logarithm of rate constant method of the reaction versus Absorbance of molar concentration of

analyte in the sample within the specific range.

Precision was checked at three concentration levels. Eight replicate measurements were recorded at each concentration level. The calculated relative standard deviation were all below 2.5% indicating excellent precision of the proposed procedures at both level of repeatability and intermediate precision.

Limit of detection (LOD*):* was calculated based on standard deviation of response and the slope of calibration curve. The limit of detection was expressed as,

$$\mathrm{LOD} = \frac{3\sigma}{S}$$

Where σ is the standard deviation of intercept s is the slope of calibration curve. The results were summarized in (Tables 3, 4 & 5) indicating good sensitivity of the proposed method. According to USP XXV guidelines, the calculated LOD values should be further validated by laboratory experiments

Limit of Quantification (LOQ*):* was calculated based on standard deviation of intercept and slope of calibration curve. In this method, the limit of quantization is expressed as

$$LOQ = \frac{10\sigma}{S}$$

The results were summarized in (Tables 3, 4 & 5) indicating the good sensitivity of the proposed method. According to USP XXV guidelines, the calculated LOQ values should be further validated by laboratory experiments.

Application to pharmaceutical dosage forms

The Fixed time method and rate constant methods of the proposed kinetic spectrophotometric method for the investigated drugs have been tested on commercial pharmaceutical dosage forms. The concentration of investigated drugs was computed from its responding regression equations. The results of proposed method (Fixed time and rate constant methods) were statistically compared with those of reported methods, in respect to accuracy and precision. The obtained mean recovery values were recorded in (Tables 3, 4 & 5), which ensures that there is no interference of other additives present in the studied formulations.

In the t- and F- tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicates good precision and accuracy in the analysis of investigated Fluoroquinolones in dosage forms.

4.0 Conclusion

The Fixed time and rate constant methods can be easily applied for determination of investigated Fluoroquinolones in pure and dosage forms that do not require elaborate treatment and tedious extraction of chromophoric compound. The proposed methods (Fixed time & rate constant method) are sensitive enough to enable determination of lower amounts of drug; these advantages encourage the application of proposed method in routine quality control of investigated analgesic drugs in industrial laboratories. Finally our methods provides advantage of improving selectivity, avoiding interference of colored and/ or are turbidity background of samples because it measures the increase in absorbance with time against blank treated similarly.

Acknowledgement

I Thank Mr. Kalsang Tharpa, research scholar Mysore University, Mysore for his

valuable guidance in carrying out this work.

Reference

1. Currie D, Lynas L, kennedy DG, Mccaughey WJ. Evaluation of a modified Ec 4 plate method to detect antimicrobial drugs. Food Addit Contam. 1998; 15: 651-660.

2. Ihrke PJ, Papich MG, Demanuellle TC. The use of fluoroquinolones in vatarinary dermitology. Vaterinary dermitology. 1999; 10: 193-204.

3. Zhang LT, Yuan ZF, Chen H, Ahou ZX, Zhou YM, Zhang AH, Liu WN, Ahang Z H. Extraction- Spectrophotometric determination of Lomefloxacin. Yaown Fenxi Zazhi. 1997; 17: 33-34.

4. Saleh GA. Redox spectrophtometric determination of Floroquinoline antibiotic dosage forms. Bull Pharm Sci (Assiut Univ). 1997; 20: 75-85.

5. Rahman N, Khan NA, Azmi SN. New kinetic spectrophotometric method for the determination of silymarin in pharmaceutical formulations using potassium permanganate as oxidant. Pharmazie. 2004; 59: 112-116.

6. Mostafa S, El-sadek M, Alla EA. Spectrophotometric determination of ciprofloxacin, enrofloxacin and pefloxacin through charge transfer complex formation. J Pharmeut Biomed . 2002; 27: 133-142.

7. Marzouq MA. Spectrophotometric Determination of Some Floroquinolones(dissertation). Assiut University; 2007.

8. Gouda BG, Seetharamappa J. Simple, rapid and sensitive spectrophotometric procedure has been proposed for the assay of Floroquinolones. Anal Sci. 2003; 19: 461-464.

9. Delgado J N, Remers WA. Wilson & Gisvold Textbook of a Organic Medicinal & Pharmaceutical Chemistry; Newyork, Lippincott- Ravan publication. 1988; 241-247.

10. The United States pharmacopoeia; 1998, 1104-2958.

11. European Pharmacopoeia; 3rd Edn, Sainte Ruffine, France; 1997, 5400.

12. Knoller J, Konig W, Schonfeld W, Bremm KD, Koller K. Application of high-performance liquid chromatography of some antibiotics in clinical microbiology. J Chromatogr. 1988; 427: 257-267.

13. Wallis SC, Charles BG, Gahan LR. A rapid and economical high-performance liquid chromatographic assay is described for norfloxacin in serum. J Chromatogr Biomed . 1995; 674: 306-309.

14. Cordoba-Borrego M, Cordoba-Diaz M, Bernabe MI, Cordoba D. Two sensitive and selective analytical methods using fluorescence (FL) and UV spectrophotometry (UV). J Pharmceu Biomed. 1996; 41: 977-982.

15. Djurajevic PT, Jelikic Stankov M, Stankov D. Fluorescence reaction and complexation equilibria between norfloxacin and aluminium (III) ion in chloride medium. Anal Chim Act. 1995; 300: 253-259.

16. Amin AA, El-sayed GO. Utility of certain π-acceptors for the spectrophotometric determination of Norfloxacin. Analyst. 1995; 120: 1189-11193.

17. El Walily AFM, Razak OA, Belal SF, Bakry RS. Determination of norfoxacin spectrophotometrically using 2,4- dinitrofluorobenzene. J Pharmceu Biomed. 1999; 21: 1069-1076.

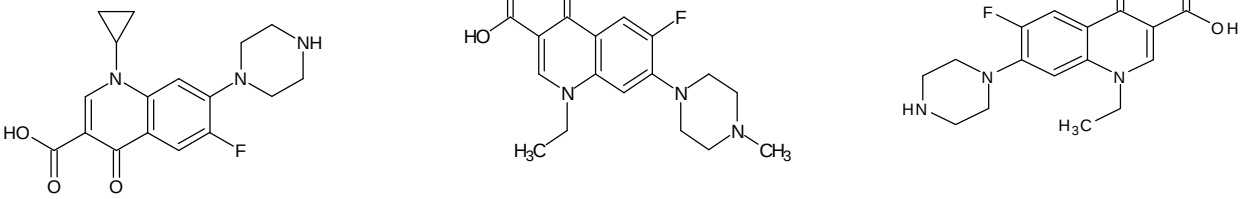
18. Suliman FEO, Sultan SM. Sequential injection technique employed for stoichiometric studies, optimization and quantitative determination of some fluoroquinolone antibiotics complexed with iron(III) in sulphuric acid media. Talanta, 1996; 43: 559-568.

19. Sastry CSP, Rao KR, Prasad OS. Two simple and sensitive extractive spectrophotometric methods for the determination of some fluoroquinolone derivatives (norfloxacin, NRF; ciprofloxacin, CPF; ofloxacin, OFL; and enrofloxacin, ERF) with Supracene Violet 3B (SV 3B, method A) and tropaeolin; Talanta. 1995; 42: 311-316.

20. Janin N, Meugnier H, Desnotters JF. Determination of Pefloxacin, a new fluoroquinolone, was given to 10 volunteers in single 400-mg oral doses repeated at 12-h intervals during 7 days. Antimicrob. 1987; 31: 1665-1668.

Figure 1: Structures of studied Floroquinolones drugs

Ciprofloxacin **Pefloxacin** **Norfloxacin**



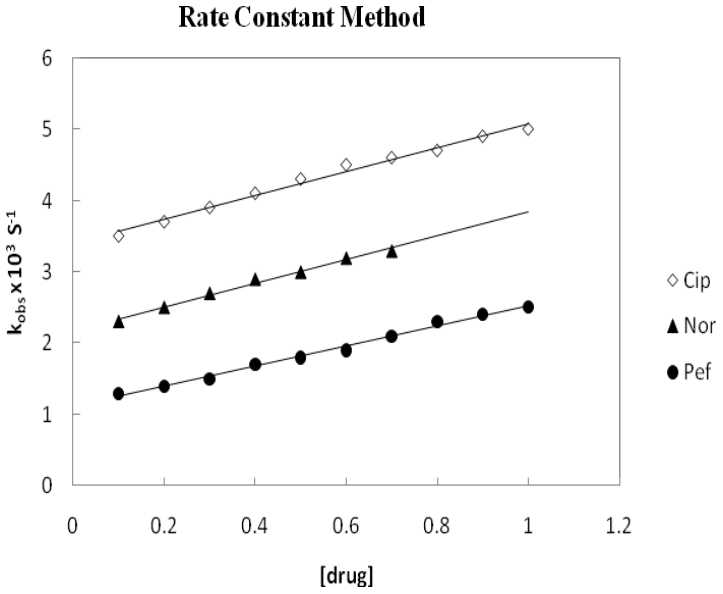


Figure 2: Calibration graph of Fluoroquinolones for Rate constant method

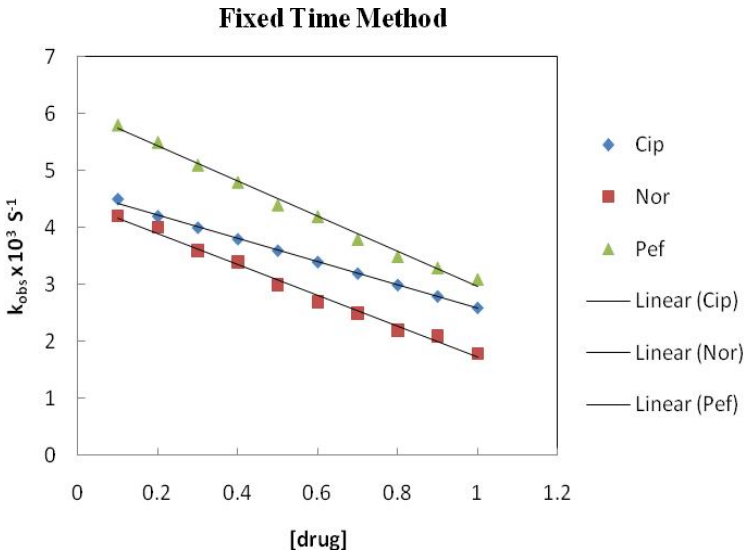


Figure 3: Calibration graph of Fluoroquinolones for Fixed time method

[Drugs]x 10^3/ mol dm^{-3}	Rate constant method K_{obs} x 10^3 S^{-1}			Fixed time method (t = 120 s) Abs.		
	Cip	Nor	Pef	Cip	Nor	Pef
0.1	5.0	3.3	2.5	4.5	4.2	5.8
0.2	4.9	3.2	2.4	4.2	4.0	5.5
0.3	4.7	3.0	2.3	4.0	3.6	5.1
0.4	4.6	2.9	2.1	3.8	3.4	4.8
0.5	4.5	2.7	1.9	3.6	3.0	4.4
0.6	4.3	2.5	1.8	3.4	2.7	4.2
0.7	4.1	2.3	1.7	3.2	2.5	3.8
0.8	3.9	-	1.5	3.0	2.2	3.5
0.9	3.7	-	1.4	2.8	2.1	3.3
1.0	3.5	-	1.3	2.6	1.8	3.1

(Cip= Ciprofloxacin, Nor= Norfloxacin, and Pef= Pefloxacin)

Table 1 .Various Kinetic methods for the determination of Fluoroquinolones.

Methods	**Drugs**	**Linear Range(µg/ml)**	**Intercept**	**Correlation coefficient(R^2)**	**LOD**	**LOQ**	**Sandwell's sensitivity**
A. Fixed time method	Ciprofloxaci	33.1-331	0.4699	0.995	0.0018	0.0006	8.6×10^{-5}
	Norfloxacin	31.9-319	0.4470	0.995	0.0040	0.0011	9.6×10^{-6}
	Pefloxacin	33.5-335	0.6079	0.996	0.0012	0.0036	6.8×10^{-6}
B. Rate constant method	Ciprofloxaci	33.1-331	0.0034	0.995	0.0164	0.0498	$8.6X\ 10^{-4}$
	Norfloxacin	31.9-223.3	0.0022	0.996	0.0828	0.2510	1.0×10^{-3}
	Pefloxacin	33.5-335	0.0012	0.998	0.0947	0.2869	$2.0X10^{-3}$

Table2: Analytical parameters of Fluoroquinolones with Acidic KMno4.

Drug	**Labelled**	**Found (X ± RSD)**	
		Proposed method	**Reference method**
Alcipro	500 mg/Tab	498 ± 0.7 t = 0.3, F = 1.3	500.9 ± 0.4
Aricip	250 mg/Tab	249.5 ± 0.6 t = 0.4, F = 1.0	249.9 ± 0.1
Cifomed	100 mg/Tab	98.0 ± 0.7 t = 0.8, F = 0.3	99.9 ± 0.2
Adiflox	3mg/Ointment	2.9 ± 0.5 mg t = 0.2, F = 1.9	3.2 ± 1.2 mg

Table 3: Analysis of Ciprofloxacin in pharmaceutical formulations

Drug	Labelled	Found (X ± RSD)	
		Proposed method	**Reference method**
Actiflox	400 mg/Tab	398 ± 0.7 t = 0.1, F = 0.5	400.2 ± 0.6 mg
Negaflox	400 mg/Tab	399 ± 0.8 t = 0.9, F = 1.4	400 ± 0.4 mg
Nifdin	100 mg/ Tab	99 ± 0.3 t = 1.2, F = 0.9	100.3 ± 0.3 mg
Norbactin	200 mg/Tab	199.2± 1.1 t = 0.2, F = 1.9	200.3 ± 0.3mg
Norflox	5 mg/Tab	4.9 ± 1.1 t = 0.2, F = 1.9	5.1 ± 0.2mg

Table 4: Analysis of Norfloxacin in pharmaceutical formulations

Drug	Labelled	Found (X ± RSD)	
		Proposed method	**Reference method**
Pefbid	400 mg	399 ± 0.5 t = 0.1, F = 0.3	400.2 ± 0.4mg
Peflobid (Inj)	100mg/50 ml	99 ± 0.8 t = 0.8 F = 1.2	102.2 ± 0.3 mg
Peze	40 mg/Tab	38.9 ± 0.4 t = 1.2, F = 0.9	39.9 ± 0.5 mg
Plx	400 mg/Tab	399.5 ± 1.1 t = 0.2, F = 1.9	399.9 ± 0.2 mg

Table 5: Analysis of Pefloxacin in pharmaceutical formulations